

**IN THE UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF OKLAHOMA**

**STATE OF OKLAHOMA, ex rel. W.A. DREW
EDMONDSON, in his capacity as ATTORNEY
GENERAL OF THE STATE OF OKLAHOMA
AND OKLAHOMA SECRETARY OF THE
ENVIRONMENT C. MILES TOLBERT, in his
capacity as the TRUSTEE FOR NATURAL
RESOURCES FOR THE STATE OF
OKLAHOMA**

PLAINTIFFS

v.

CASE NO.: 05-CV-00329 GKF –SAJ

**TYSON FOODS, INC., TYSON POULTRY, INC.,
TYSON CHICKEN, INC., COBB-VANTRESS,
INC., CAL-MAINE FOODS, INC., CAL-MAINE
FARMS, INC. CARGILL, INC., CARGILL
TURKEY PRODUCTION, LLC, GEORGE'S,
INC., GEORGE'S FARMS, INC., PETERSON
FARMS, INC., SIMMONS FOODS, INC. and
WILLOW BROOK FOODS, INC.**

DEFENDANTS

Report by Dr. Samuel Myoda

1. Introduction

- 1.1. See attached CV for background information, publications, education, employment history and credentials for Dr. Myoda.
- 1.2. IEH was retained in November, 2007 by Defendants at an hourly rate of up to \$400/hr to serve as expert consultants regarding, but not limited to, microbial source tracking, microbiology, water quality, public health and related regulatory affairs. IEH has been asked to review the Plaintiff's testimony and reports, the water quality data and other relevant parameters that influence water quality in the Illinois River Watershed (IRW) and to offer our scientific opinions on the water quality and factors that affect the water quality in the IRW.
- 1.3. We previously submitted an affidavit, testimony and demonstratives in this matter. We incorporate by reference my previously stated opinions as amended.

2. General Microbiology

- 2.1. Bacteria are microscopic, unicellular organisms that are prokaryotes, meaning that unlike our cells they do not contain a nucleus. There are many different physical and biological properties of these organisms, they have preferred habitats, and interact with their surroundings in many different ways. There are literally trillions upon trillions of bacteria in the environment and the overwhelming majority of them are not pathogenic. Bacteria are an integral part of our lives; they are used in food production, e.g. yogurt and cheese, in our digestive system, e.g. *E. coli* and enterococcus, in the soil, e.g. nitrogen fixing bacteria that are essential to the nutrient cycle and are also in the air and water. Bacteria are used to treat wastewater, to break down pollutants, e.g. bioremediation, and are used in drug development and production. There are a plethora of beneficial uses for bacteria.

2.2. Despite the wealth of knowledge regarding bacteria, in fact relatively little is known about the total universe of bacteria, and it is estimated that scientists have been able to culture (grow in the lab) less than 2% of the bacteria that exist. There are a host of factors that affect the “fate and transport” of specific types of bacteria in the environment: how do different bacteria handle different environmental factors; what is the relationship between bacterial transport and precipitation; what factors govern soil infiltration and filtration/sorption; what characteristics govern relative survival rates, predation rates, and growth rates; and the impact of factors such sunlight (UV radiation), temperature, sedimentation, humidity, pH, moisture content, etc.? These factors affect the fate and transport of each bacterium.

3. Indicator Based Water Quality Standards to Protect Public Health

3.1. Developing strategies to protect public health has driven the study of microbiology. In the late 1800s/early 1900s, illness due to poor sanitation and water quality was commonplace, largely due to inadequate sewage treatment, hence the need to develop and install sewage treatment facilities and limit if not prevent fecal matter from entering the water. In order to evaluate the effectiveness of the treatment interventions and resulting water quality, a test was needed. The ideal approach is to test for the pathogens in the water directly; however, many of the pathogens were unknown, in relatively small quantities and hard if not impossible to test for at the time. Therefore, the indicator organism approach was embraced and because certain coliforms are virtually always found in feces, the presence of total coliforms was used as an indication that fecal contamination was probable. The total coliform group contains bacteria such as *Citrobacter*, *Enterobacter*, *Escherichia* (*E. coli*), *Hafnia*, *Klebsiella*, *Serratia* and *Yersinia*.

3.2. The problem with using total coliforms as an indicator of fecal contamination is that not all the bacteria in this group are found in feces. In an effort to increase the accuracy of the indicator organism approach, a subset of total coliforms – the faecal coliform group (aka fecal coliform) replaced total coliforms as a measure of water quality. The faecal coliform group contains bacteria such as *E. coli*, *Citrobacter*, *Enterobacter* and *Klebsiella*. Although this was a better approach, it still did not eliminate the possibility that indicators would be found when in fact there was no fecal contamination (or pathogens) present. This is because in the faecal coliform group, *E. coli* is associated with feces however; organisms such as *Citrobacter*, *Enterobacter* and *Klebsiella* do not originate in feces and because the faecal coliform count does not differentiate which bacteria(s) are being quantified, it is not known which organisms are present/absent or where they came from.

3.3. In the early 1980s, realizing the shortcomings of the use of faecal coliforms as an indicator, the EPA set out to develop a better methodology to measure water quality. The technology was still not in place to directly detect all the potential pathogens so alternative indicator bacteria were evaluated. By definition, indicator(s) must be easy to detect, non pathogenic, exist in greater quantities than the pathogen and must live longer than the pathogens. An indicator would be useless if it did not persist in the environment at least as long as the pathogen. In addition, there should be a correlation of the indicator concentration with the pathogen concentration and the fate and transport properties of the indicator and pathogen ideally should be identical but at a minimum need to be correlated. The EPA did not at that time evaluate the correlation of indicator with pathogen or the fate and transport characteristics of each. The EPA did, however, evaluate the indicator bacteria concentration(s) versus number of reported gastrointestinal illnesses reported by swimmers using the water bodies for primary contact recreation although they did not attempt to identify which pathogens were causing

illnesses. The general consensus in the scientific community is that the majority of the illnesses were caused by enteric viruses; the evidence supports this conclusion as the studies were done at beaches that were impacted by human wastewater discharges that would typically carry human enteric viruses. The conclusions of the epidemiological studies resulted in issuance of the *Ambient Water Quality Criteria for Bacteria -1986* (EPA440/5-84-002) that recommended a water quality standard of a geometric mean of 126CFU *E. coli*/100ml (235CFU/100ml to 576CFU/100ml single sample maximum) or 33 CFU enterococcus/100mL (61CFU/100ml to 151CFU/100ml single sample maximum) for fresh water and 35CFU enterococcus/100ml (104CFU/100ml to 500CFU/100ml single sample maximum) for marine water (the EPA determined that the risk levels associated with these indicator bacteria concentrations are between 8 to 19 illnesses per 1,000 swimmers). After its issuance, the EPA recommended that all States use either *E. coli* or enterococcus instead of faecal coliforms as indicators of water quality and in 2000, the Beaches Environmental Assessment and Coastal Health (BEACH) Act required States adjacent to the Great Lakes and coastal states to adopt the 1986 Standards.

3.4. The recommendation to adopt the federal guidelines was met with resistance due to the reluctance to change for a variety of reasons, including but not limited to the fact that the correlation of illnesses to indicator concentrations was not as strong as some deemed appropriate and that the epidemiological studies were carried out in waters that were impacted by wastewater treatment plant discharges. One of the major objections was that in areas that have wastewater treatment plant effluent the illness rate versus indicator concentration would be higher due to the presence of human enteric viruses in the effluent. At the time of the studies, the EPA's intention was to do additional studies to determine if indicators that were derived from various sources did in fact hold a different correlation with illness rates. However, the studies were never carried out due to funding constraints. The

indicators, both *E. coli* and enterococcus are shed from virtually all warm blooded animals, e.g. cattle, pigs, deer, birds, wildlife, waterfowl, humans, pets, etc. In fact, wildlife, waterfowl and birds are major contributors of the *E. coli* and enterococcus that are found in surface waters. The issue of different sources was addressed in the EPA 1994 *Water Quality Standards Handbook* that allowed a State to discount all indicator bacteria derived from nonhuman sources when making regulatory decisions. This policy was extremely important because States were in the process of developing total maximum daily load regulations (TMDLs) to address high levels of bacteria in surface waters throughout the country and finding that *E. coli* and enterococcus were ubiquitous in the environment. A TMDL is the regulatory mechanism that is in place to address impaired waters. The TMDL process includes but is not limited to monitoring, identification of the sources of a pollutant, assignments of load and waste load allocations, pollution control strategies, public participation, and permitting coordination (MS4, CAFO and NPDES) to remediate the problem. The State of Oklahoma has not promulgated a TMDL for the IRW. The appropriate first step to addressing water quality concerns in the IRW should have been the development of a TMDL, not litigation.

4. EPA Development of Revised Water Quality Standards for Bacteria

4.1. In 2004, the EPA promulgated a rule that required the States included in the 2000 BEACH Act to adopt the 1986 bacteria standards and reversed the policy of discounting nonhuman derived bacteria indicators. Also included in the 2000 BEACH Act was a mandate that the EPA reevaluate the standards and develop revised standards by 2005. However, the EPA failed to meet this deadline. In spring of 2007 the EPA convened the Experts Scientific Workshop to discuss the critical research and scientific needs relating to the development of the new recreational water quality standard; Dr. Myoda was invited and served as an expert at this

workshop. A five year timetable was mapped out for completion of the revision of the standards culminating in the adoption of revised water quality standards by 2012. One of the major issues addressed in the workshop was the problem that there was little or no correlation between the current indicator organisms and many of the human pathogens. Furthermore, any relationship between the fate and transport characteristics of the indicators versus the pathogens is dependent upon the source of the indicators and the nature of their environment. Therefore, the correlation between indicator concentrations and risk levels is unclear. The panel considered areas impacted by agricultural sources to be of the highest priority for research because the inadequacy of the present indicator approach, i.e. *E. coli* and enterococcus, was evaluated to be greatest in those areas. Poultry was differentiated from other agricultural animals because the risk level from poultry was considered to be lower than that of other agricultural sources, e.g. cattle.

- 4.2. When developing the appropriate indicator(s), knowledge of the fate and transport characteristics of the indicator(s) and pathogens, both individually and as they relate to each other, is critical. Individually, fate and transport is significant because only those pathogens that are present and viable in the water pose a potential public health risk to those recreating in the water. As the microbiological characteristics of each pathogen are significantly different, it is highly likely that their fate and transport characteristics will vary as well. Ideally, the indicator(s) chosen as the surrogate for the pathogens will have the same fate and transport characteristics of the pathogens themselves. However, since this is unlikely, it is important to know and relate the characteristics that are indicator(s) specific to the pathogens so that the measurement of the indicator can be correlated to the concentration of the viable pathogens in the water and ultimately to public health risk.

4.3. The most simplistic route of transport is direct deposition, e.g. cattle defecating in streams.

Once the pathogen(s) (assumed to be carried in the feces of warm blooded mammals) is excreted over or in the water, the relevant questions are how long the pathogen will be viable and available in the water column. Indirect deposition of feces introduces many more variables affecting the fate and transport of the bacteria and/or pathogen. First, the fecal properties from different mammals vary substantially. One of the primary differences (aside from pathogen and indicator density) is moisture content. Very “wet” feces is more likely than “dry” feces to introduce pathogens into the environment. After defecation, the distance from the water plays an important role as well. Driven by heavy precipitation and transported primarily via surface runoff, the organisms may be washed into the surface water by sheet flow where it occurs. During this transport, they are subjected to a variety of environmental factors including but not limited to UV disinfection, predation, temperature etc. that affect the proportion that will ultimately end up in surface water in which people are recreating. The application of poultry litter is an example of indirect deposition. The indicator bacteria (as well as any other bacteria contained in the feces) are subjected to the conditions in the litter for great lengths of time prior to the application of the litter as a fertilizer/soil amendment. During this time, the composting processes along with natural die off kill a substantial portion of the bacteria. What bacteria may survive until application is then subjected to the aforementioned environmental factors and only a small portion (if any) will remain viable.

4.4. Resuspension from sand or sediment could also play an important role in contributing to the indicator organism concentrations in the water column. There may be a reservoir of indicator(s) that could be reintroduced into the water column. Additionally, regrowth of the indicator(s) could represent a source and confound the risk assessment/prediction.

5. Microbial Source Tracking (MST)

5.1. The science of microbial source tracking (MST) was developed in the early/mid 1990s during which time the EPA policy allowed the discounting of nonhuman sources of indicator bacteria. MST was conceived as a tool to discriminate between human and the nonhuman sources so that appropriate TMDLs, pollution control strategies (PCS) and best management practices (BMPs) could be developed. MST was an extension of the principles used in track down studies such as those done by the CDC during an illness outbreak. Antibiotic resistance analysis (ARA, aka ARP – antibiotic resistance pattern) was one of the more widely used MST techniques. Initial studies reported high average rate of correct classifications (ARCC) as a measure of accuracy (using various calculation techniques including the holdout method of cross validation) and suggested that an ARCC of 60% to 70% was enough for water quality managers to base decisions on (Harwood et. al, 2000, *Classification of Antibiotic Resistance Patterns of Indicator Bacteria by Discriminant Analysis: Use in Predicting the Source of Fecal Contamination in Subtropical Waters*). Encouraged by the results of initial studies and due to the time constraints relating to the development of TMDLs and other regulatory pressures, ARA was quickly embraced by many as a mainstream technology and was widely used for MST. But as more studies were undertaken and ARA as well as other MST methods were challenged by the scientific community in method comparison studies in which known samples were given blindly to participating labs, the majority of the methods performed poorly. In both the Southern Coastal California Water Research Project (SCCWRP)/EPA study and the USGS method comparison studies, ARA performed very poorly and had extremely high false positive rates (39% to 100%). Dr. Harwood participated in the SCCWRP study and utilized this methodology; her lab's performance reflected this deficiency. In addition, it was determined that the use of ARCC using techniques such as the holdout method of cross validation was not an adequate

measure of the accuracy of the method (Harwood et. al., 2004, *Phenotypic library-based microbial source tracking methods: Efficacy in the California collaborative study* and Stoeckel and Harwood, 2007, *Performance, Design, and Analysis in Microbial Source Tracking Studies*). In general, library-based methods performed poorly, especially those that employed the population ecology approach to fingerprint analysis. The population ecology approach matches genetic patterns, e.g. ribotyping “fingerprints” by using mathematical algorithms to estimate the similarity between two fingerprints. In reality, two fingerprints can be very similar, often differing by only one band and be from bacteria originating from different sources. One method that is much more accurate is the molecular epidemiological approach in which only identical matches are considered to originate from the same source. This approach is used by the CDC and other regulatory agencies in track down investigations such as the determination of the source of disease outbreaks. IEH uses this analytical approach and performed the best in these method comparison studies.

- 5.2. It is imperative that standard methods that have been accepted by the scientific community are followed. These methods should be approved by the appropriate authority such as the EPA, Standard Methods for the Examination of Water and Wastewater, and/or AOAC, etc. This ensures that, if the tests are carried out correctly, the results are reliable and reproducible. In addition to utilizing the proper testing method, sample collection must be carried out with equal rigor and quality controls. Issues such as hold time (normally included in the standard method) must be strictly adhered to or the results are invalid, e.g. exceeding the hold time on water samples that are being analyzed for bacteria concentrations could lead to higher counts due to regrowth. Statistically valid sampling plans must be followed; sample locations and the time the samples are taken must be randomly selected. A minimum number of samples must be taken to ensure that the testing reflects an accurate picture of the whole. Positive and

negative controls must be used. Unless all these elements are included in a scientific study, the results are questionable if not invalid.

6. 303(d) Listings in the IRW

6.1. Relative to the EPAs recommended standards; surface waters throughout the country are out of compliance. For example, Delaware is a state with three counties, one primarily urban, one primarily agricultural and one mixed. Delaware lists approximately 97% of the State's waters on the 303(d) list as impaired due to high enterococcus levels. Coincidentally, Delaware monitors approximately 97% of its surface waters. Throughout the country, the single largest cause of impairments resulting in 303(d) listings as "impaired waters" is pathogens (http://iaspub.epa.gov/waters10/attains_nation_cy.control?p_report_type=T).

In Oklahoma, 5,847 miles of stream segments are listed as impaired due to high enterococcus levels, 3,118 miles due to high *E. coli* levels and 2,921 miles due to high faecal coliform levels. Of the lakes assessed, 34% did not meet the primary contact recreation standards (Tenkiller Ferry Lake is not impaired by bacteria). In Oklahoma, more stream segments are listed as impaired for enterococcus than any other water quality parameter. In the IRW there are no

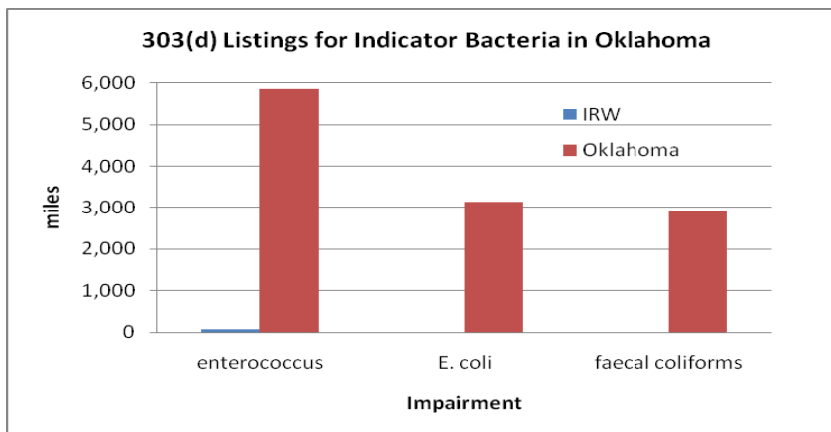


Figure 1. 303(d)listings in Oklahoma

stream segments listed as impaired by faecal coliforms, 6.2 miles listed as impaired by *E. coli* and 73.5 miles listed as impaired by enterococcus. This represents 0% of Oklahoma's impairments for faecal coliforms, 0.2% of Oklahoma's impairments for *E. coli* and only 1.26% of Oklahoma's impairments for enterococcus (**Figure 1**, Oklahoma 2006 303(d) list). Throughout the USA, there are 10,294 segments listed as impaired for bacteria (**Figure 2**).

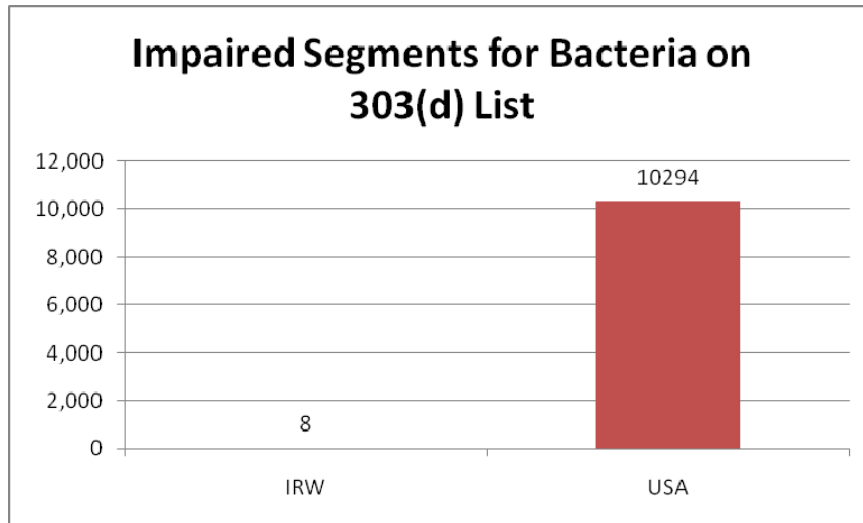


Figure 2. 303(d) Listings for Bacteria in the USA

6.2. All of the watersheds in Oklahoma contain waters that are listed as impaired and require TMDLs. The issue of high bacteria levels is prevalent throughout Oklahoma and is in no way confined to the IRW or areas that are used for poultry production or the application of poultry litter. The bacteria levels that are seen in the IRW are typical of the levels seen throughout Oklahoma as well as the rest of the country as evident by 303(d) listings and TMDLs that require reductions of up to and in some times greater than 90% of the indicator bacteria.

7. Supplemental Water Quality in the IRW

7.1. In reviewing the data it is apparent that standard methods were not followed in the Plaintiffs' testing. During our initial review, we looked at approximately 1,700 water sample records and

over 72% exceeded the 6 hour hold time mandated by the EPA for recreational water being tested for indicator bacteria (*E. coli*, enterococcus) was violated, in many cases by one to two days. In no instance did the Plaintiffs exclude data derived from samples that did not meet the 6 hour hold time. Therefore, the conclusions based on these data are unreliable. In addition, the primary body contact recreational water quality standard is evaluated against a geometric mean of no less than 5 samples equally spaced over a 30 day period, a frequency that was virtually never achieved during the Plaintiffs' study, neither in the data used by the State nor any and all supplemental data sets. Sampling locations were not chosen randomly. It appears that the locations that the Plaintiffs thought it most likely to find what they wanted to find were chosen. Furthermore, it appears that the timing of sample collection was not randomized either, both with respect to the time of day samples were collected and the timing relative to flow conditions. Many of the samples were taken during high flow conditions during which bacteria counts will generally be higher than average due to resuspension and runoff. Flow conditions are normally accounted for in a TMDL so that an accurate model or representation of the watershed can be used to assess water quality. There are many ways to consider flow e.g., a hydrodynamic model, flow duration curve, cumulative distribution, etc. Flow was not considered in the Plaintiffs' analysis. In fact, many of the supplemental samples were taken during high flow events resulting in data that reflects an artificially high level of indicator bacteria in the IRW. Based on all of these violations of standard methodologies, we believe that the data is unreliable and is biased and skewed in favor of the Plaintiff's position.

7.2. The CRA report revealed egregious violations in proper sampling protocols. These violations included but were not limited to samplers walking through feces into water that they then sampled, soil borers driven through feces and into the dirt when soil samples were taken and between uses, sampling tools were not disinfected. A review of the edge of field sample data

reflects that the mean bacteria concentration for *E. coli* is 4,174 CFU/100ml, for enterococcus is 14,664 CFU/100ml, and for faecal coliforms is 6,371 CFU/100ml. Although there were a few samples reported to have concentrations of 1,600,000 CFU/100ml, those are atypical and represent outliers in the data set. However, even those outlying values are an order of magnitude below that of sewage influent (5,400,000 CFU *E. coli*/100ml, Miyanaga et. al, 2006, *Detection of Escherichia coli in the sewage influent by fluorescent labeled T4 phage*). In my view, the atypically high values are more consistent with samples taken in close proximity to a concentrated source of indicator bacteria, e.g. cattle feces, than with runoff samples taken from areas affected by uniformly distributed indicator bacteria such as the application of poultry litter.

7.3. The indicator bacteria in the waters of the IRW originate from many sources. The loading from cattle is extremely significant. Typically, cattle will excrete 15 to 35 kg of feces per day. In the summer when the majority of primary contact recreation is occurring, the initial *E. coli* concentration in the cattle feces will be approximately 3,000,000 CFU *E. coli*/gram. However, after deposition the bacteria multiply and reach levels up to 48,000,000 CFU *E. coli*/gram (Sinton et. al, 2007, *Survival of Indicator and Pathogenic Bacteria in Bovine Feces on Pasture*). Also in the summer months the cattle tend to congregate near and in the streams in order to cool off, increasing the possibility of direct deposition into and in close proximity of the streams. This means that each day one cow will contribute roughly 960,000,000,000 *E. coli* into the environment and with approximately 200,000 head of cattle in the IRW over 192,000,000,000,000,000 CFU *E. coli* will be introduced into the environment each day. Contrasting the growth of indicator bacteria in a "cow pie" is the death of indicator bacteria in the litter via composting in the poultry houses and by direct exposure to UV radiation. The litter is spread in a very thin layer on the surface of a field, it is dry and the indicator bacteria

are not protected as they are in “cow pies”. Dr. Harwood testified that she believed that the indicator bacteria in poultry litter would only survive for a few hours after application.

7.4. In addition to cattle, there are approximately 150,000 swine and numerous wildlife (geese, ducks, deer, turkeys, other birds, small mammals, rodents, etc.) that live throughout or migrate through the watershed. The wastewater treatment plant effluent and septic system loads are additional sources of indicator bacteria. As indicated in the numerous TMDLs that have been established in OK, wildlife are significant sources of fecal material and indicator bacteria. The USGS reported that in Delaware County, Oklahoma, 45% of the *E. coli* sampled came from birds and 22% came from cattle (*Reconnaissance of the Hydrology, Water Quality, and Sources of Bacterial and Nutrient Contamination in the Ozark Plateaus Aquifer System and Cave Springs Branch of Honey Creek, Delaware County, Oklahoma, March 1999–March 2000*).

8. Pathogenic Bacteria Not Detected in the IRW

8.1. The presence of indicator bacteria does not mean that pathogens are present. The pathogens that the Plaintiffs claim to be present include salmonella, campylobacter and *E. coli* O157 (a strain of *E. coli* that is pathogenic to humans). These pathogens are carried by a variety of hosts. For example, *E. coli* O157 are primarily found in cattle, salmonella in reptiles and poultry, and campylobacter in cattle, swine and poultry. Other hosts could carry these pathogens as well. The Plaintiffs contend that *E. coli* O157 is shed from poultry. However, there is virtually no evidence that poultry carries *E. coli* O157 and the Plaintiffs never tested for or found it in the litter or in the environment. Campylobacter does not survive well in the environment. It will die when exposed to oxygen and will also readily dehydrate and die. We find the Plaintiffs’ campylobacter testing results in the IRW not surprising. No campylobacter was found in litter, ground water, dust, soil, public water supply or spring water samples and

only 2 out of 302 surface water samples contained campylobacter. We find the Plaintiffs' salmonella testing results in the IRW not surprising as well. No salmonella was found in ground water, dust, soil, public water supply or spring water samples and only 24 out of 562 surface water samples and 2 out of 17 litter samples contained salmonella. In both the salmonella and campylobacter positive samples, the source(s) were not identified.

The basic precept in the field of regulatory control of the microbiological status of water and environmental samples is the identification and enumeration of viable indicator organisms and pathogens as the only predictor of the public health status of a specific material. A wide spectrum of specific tests and criteria has been developed to grow and enumerate microorganisms in the laboratory setting. The specifics and usages of these analyses are listed in the Federal Register and have been published in the Standard Methods for the Examination of Water and Wastewater. Using the results from these testing procedures, US EPA and state and local regulatory entities enforce the standards set up by the appropriate governing bodies. It is only through the use of such scientifically sound and verifiable testing criteria that effective enforcement can be accomplished for the public's benefit.

The concept that some potentially pathogenic microorganisms may be present in the environment but are in a metabolic state from which they cannot be grown in the laboratory (viable but not culturable or VBNC) has been reported. How a situation concerning VBNC organisms in the environment may impact human health is a matter of dispute. In some studies, the appropriate application of suitable culturing conditions have been questioned, cells subsequently observed were from a very minor subpopulation viable cells (2, 4, 9, 11) or cells adapted to utilize the nutrients released from dead cells (3) in the original sample. Some studies have used tests and assays that measure both live and dead cells, never reflecting on the fact that the dead cells posed no threat to humans. Resuscitating VBNC organisms often

takes non-physiological conditions, referred to by one researcher as “exquisite laboratory conditions” (8). Winfield and Groisman state, “Recovery from a VBNC state occurs rarely, if at all” (17). Studies using VBNC cells directly indicated that pathogenicity was not seen (4, 5, 6, 8, 10, 13, 14, 15, 17). In fact some researchers consider the concept of ‘viable but not culturable’ as an oxymoron (1). Barer and Bogosian (2004) summarize the metabolic status of VBNC cells, stating, “that the observed nonculturable cells are either dead or passing through a brief injured state to death.” For effective regulation and enforcement in monitoring environmental samples for potential health threats, there is a necessity for verifiable identification of microorganisms in a metabolic state and in sufficient numbers to initiate a disease process in humans. No evidence exists to support such conditions arising from VBNC organisms in the environment. The microbiological regulatory field is based on the enumeration of viable organisms and not on detecting the presence of material that that does not pose a threat to human health. Plausible predictions on the safety of food, potable water, and wastewater effluent would be impossible without reliable and reproducible assays to quantify the actual levels of viable suspect organisms in these materials.

The Plaintiffs’ explain that the negative results of their tests for pathogenic salmonella and campylobacter are due to the fact that these organisms are present but in the VBNC state. This is not true. The Plaintiffs’ did not find them because they were not present. The lack of these pathogens and confirmation of their negative results could have easily been confirmed using molecular methods. In fact, e-mail correspondence and lab a notebook entry indicates that some samples were tested for pathogens using PCR. However, we were unable to find any of those results in the material that the Plaintiffs’ provided and Dr. Harwood indicated in her deposition that she did not use PCR to test for pathogens. We find it very puzzling that the written records do no match her testimony. In addition, in support of the Plaintiffs’ VBNC

theory, Dr. Harwood's report states that "Many studies have indicated that pathogens which enter the VBNC state remain infectious (Baffone et al., 2003; Oliver and Bockian, 1995) including *Campylobacter jejuni* (Baffone et al., 2006) and *E. coli* O157:H7 (Makino et al, 2000)."

This is very misleading. Baffone et al., 2003 reports that cells from 3 strains of *Vibrio* were incubated in salt water until their viable counts were $>0.1\text{cfu/ml}$. 0.1 ml was used to infect mice per gastric. Subsequently mice were sacrificed and the gut tissue cultured. Recoveries of the inoculated *Vibrio* strain were seen in 25-50% of the mice. It took 2 sequential mouse passages for the organisms to recover pathogenic characteristics. *Vibrio* is not found in chickens, it is a pathogen of concern in shellfish. Baffone et al., 2006 reported that using similar inoculation criteria with different strains of campylobacter, it was isolated from less than 30% of mice tested. None were deemed 'infected' as was stated in the Harwood report – rather we can only say that they were 'resuscitated'. Furthermore, $\sim 10^4$ metabolically active cells (though 'nonculturable') were needed to effect subsequent recovery of campylobacter from the mouse gut. This number is significantly higher than what is needed for normal metabolically active cells and is significantly higher than concentrations typically found in the environment.

9. Plaintiffs' Flawed "Biomarker"

9.1. Plaintiffs purport to have identified a poultry specific "biomarker", which they allege allows them to specifically identify bacterial contamination derived from poultry sources. Plaintiffs further assert that their process allows them to quantify the amount of "biomarker" which they suggest correlates with the presence of indicator bacteria. The development of the Plaintiffs' "biomarker" process was deeply flawed in many ways. In fact, their conclusion that their "biomarker" is specific to poultry is demonstrably false.

9.2. As indicated and as I discussed in my prior affidavit and testimony, the process that the Plaintiffs followed was inadequate and plagued with omissions and shortcuts. These shortcomings included: omission of feces testing; failure to investigate if any relationship exists between the “biomarker” and indicator bacteria in the feces; failure to investigate the temporal relationship between the “biomarker” and indicators in the litter; failure to isolate the organism carrying the genetic sequence amplified by the LA35 primer set; failure to characterize the fate and transport characteristics of the “biomarker; failure to prove specificity, both by inadequate sampling size and omissions of potential alternative sources of fecal matter that carry the “biomarker”; and failure to demonstrate a relationship between the “biomarker” and pathogens associated with poultry.

9.2.1. Failure to test poultry feces directly: The Plaintiffs’ central allegation is that indicator bacteria and therefore pathogens derived from poultry feces pollute the waters of the IRW. Therefore, the logical, first step in the development process should have been the identification and isolation of the “biomarker” in feces. This would have confirmed/refuted that the “biomarker” originated from poultry feces and did not originate from other sources such as the bedding material or feed.

9.2.2. Failure to investigate if any relationship exists between the “biomarker” and indicator bacteria in the feces: Had the Plaintiffs demonstrated that the “biomarker” did originate in the poultry feces, Plaintiffs could also have investigated whether there existed any relationship between the indicator bacteria (*E. coli* and enterococcus) and the “biomarker” in the feces – in this case, the Plaintiffs did neither. Therefore, neither a correlation to indicator levels nor to potential public health risk has been established in the faecal matter excreted by poultry.

9.2.3. Failure to isolate the organism carrying the genetic sequence amplified by the LA35

primer set: The plaintiffs' did not positively identify, culture or isolate the organism(s) that carry the genetic sequence that correspond to their "biomarker". In fact, the Plaintiffs did not determine if the "biomarker" was derived from a fragment of DNA from dead bacteria or from living bacteria. The genetic sequence used as the biomarker is a portion of the 16S rRNA gene. The known bacterium that has the closest sequence to this fragment is *Brevibacterium avium*. *Brevibacterium avium* was first identified and isolated from bumble-foot lesions in domestic fowl in 1999. *B. avium* can be easily cultured and is differentiated from other *Brevibacterium* species by both its genetic sequence and phenotypic traits e.g., temperature at which it grows, utilization of arabinose, etc. so it is puzzling why the Plaintiffs did not try to isolate it. *Brevibacterium avium* is considered to be nonpathogenic and has been associated with the bumble foot lesions in poultry. In general, the conditions in the intestinal tract of poultry are not conducive to *Brevibacterium sp.* survival. In addition, there are many species and strains of *Brevibacterium sp.* that can be found throughout the environment, e.g. cheese, soil, ear canal, rice, etc. By not isolating a pure culture of the organism in question, the Plaintiffs not only failed in determining if the genetic material was derived from live or dead bacteria but also forewent the opportunity to specifically identify the organism. Because they failed to do so, the Plaintiffs cannot adequately determine if the organism is specific to poultry nor can they investigate the fate and transport characteristics of the organism.

9.2.4. Failure to investigate the relationship (static and temporal) between the "biomarker"

and indicators in the litter: Regardless of the origin of the "biomarker", once in the litter, the relationship between the indicator bacteria (*E. coli* and enterococcus) and the "biomarker" should have been investigated. In order for the Plaintiffs' proposed method

to be a useful MST tool, this relationship must be clearly defined – it was not. Without defining this relationship there is no way to calculate the amount, if any, of the indicator bacteria found in the environment which originated from poultry litter. Dr. Harwood did claim to demonstrate this relationship; however, her analysis was flawed. First, no temporal relationship was established. Plaintiffs' process was developed in the first instance based on two litter samples taken on the same day from a single location. In order to establish a meaningful relationship, multiple samples from the same poultry houses must be taken over a period of time. Designing the sampling plan in this way would allow one to determine if the die-off, growth and deposition rates of the "biomarker" was related to the indicator bacteria. Because this temporal relationship was not studied, even if a correlation may be evident at one point in time, it is impossible to know if that relationship is stable. For example, if on day one the "biomarker":indicator ratio was 20:1 and the "biomarker" was growing and the indicator bacteria were dying (as would be a reasonable assumption for *Brevibacterium*:*Enterococcus*), the next week the ratio could be 100:1. Therefore, no meaningful relationship between the two can be inferred or has been established.

9.2.5. The second flaw in Dr. Harwood's attempt to demonstrate the relationship of "biomarker" versus indicator bacteria was with the correlation that she proposed. Dr. Harwood had many litter samples available yet choose to run qPCR initially on only 9 samples. Of these 9 samples, 3 had enterococcus values reported only as being greater than 120,000 CFU/g. This means that the enterococcus concentration of these samples is not known so any value assigned to them would be arbitrary. These samples should be excluded from any appropriate correlation. In addition, the time period between when these samples were taken and enumerated was not consistent. This introduced another unaccounted for

variable. Setting this aside (for the sake of illustration only), when the remaining “biomarker” concentrations are compared to the enterococcus concentrations in the remaining 6 samples there is no correlation ($r^2 = 0.1671$, **Figure 3**).

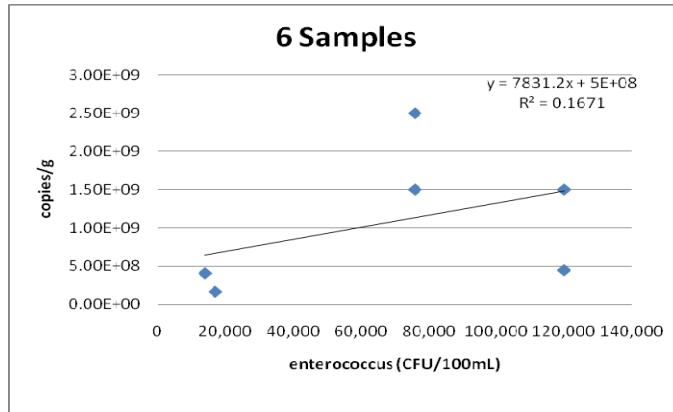


Figure 3. Six Original Litter Samples

At this point in the research, the next logical step would be either to stop because there was no correlation or assume that the sample size was too small and to test many, many more samples. The Plaintiffs did neither. They tested only one more sample, FAC1-6-20-06. FAC1-6-20-06 is very problematic. First, it was listed as a soil sample, not as a litter sample on the chain of custody form, the lab log in form, and the Certificate of Analysis. Second, FAC1-6-20-06 was not provided by the Plaintiff to the Defendants along with the initial sample set sent during discovery but was only sent after a specific request for it was made. Third, when it arrived at IEH, it was still labeled as a soil sample and did not look like a typical litter sample; it was very fine particles that resembled dark sand. Fourth, the reported enterococcus values of FAC1-6-20-06 were atypical, they were substantially lower (1 to 2 orders of magnitude) than that of other the other litter samples. It is very convenient that with the addition of this last and questionable sample, the “biomarker” versus enterococcus correlation was improved. ($r^2 = 0.2914$, **Figure 4**). However, even

considering the increased r^2 , the correlation between “biomarker” and indicator bacteria is not strong enough to demonstrate any connection between poultry litter and indicator bacteria in the IRW.

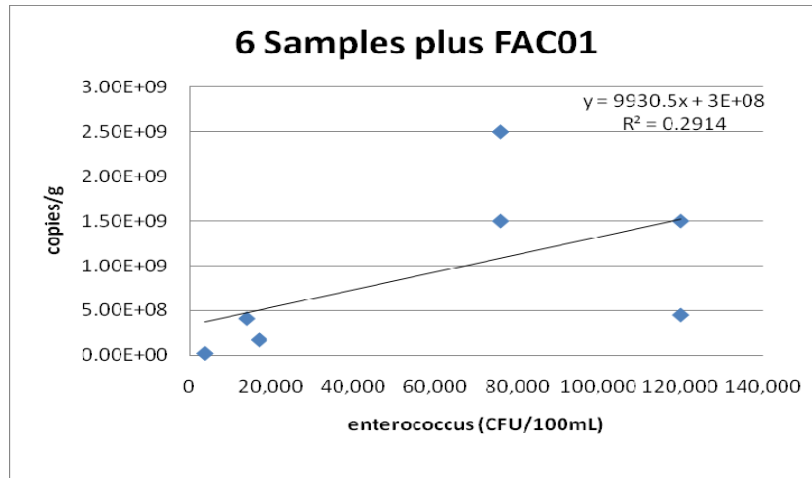


Figure 4. Six Original Litter Samples Plus FAC01

9.2.6. **Failure to characterize the fate and transport characteristics of the “biomarker:** It is crucial that a relationship between a “biomarker” and the indicator be established in order to develop any risk relationship. There are many factors that not only affect the survival of the organisms but how they travel in the environment (see Section 4). The Plaintiffs have not identified the source of the genetic material that corresponds to the “biomarker” let alone characterized the fate and transport of “biomarker”. The Plaintiffs’ method is based on the amplification of a particular genetic sequence that may or may not be from a live organism. The polymerase chain reaction (PCR) cannot differentiate between live or dead organisms. DNA can persist for long periods of time in the environment, creating positive PCR results long after the indicator or pathogen has been introduced into the environment and has died. The FAC1-6-20-06 sample that the Plaintiffs provided IEH is an excellent example, it was over two years old and had an extremely high PCR signal, demonstrating

the prolonged persistence of the target DNA in the environment. This same sample had very little enterococcus. Therefore, the public health risk would be greatly overestimated based on the “biomarker” concentration. Without knowing the fate and transport properties of the “biomarker”, it is impossible to link the “biomarker” with indicators that may also be present in poultry litter. The fate and transport of the “biomarker” was not studied, therefore, no association with poultry litter may be developed.

9.2.7. *Failure to prove specificity, both by inadequate sampling size and omissions of potential*

alternative sources of fecal matter that carry the “biomarker”: In order for a marker or signal to be an effective MST tool it must be source specific. The “biomarker” that the Plaintiffs developed and claim to be poultry specific is not specific to poultry. The Plaintiffs only tested 24 cattle manure composites, 2 swine manure composites, 10 duck and 10 goose manure composites, 3 septic and 3 WWTP samples to validate the specificity of the biomarker. In this extremely limited sampling, the biomarker was found to be carried by ducks, geese and cattle. The vast majority of the potential sources of the indicator bacteria in the IRW were not tested, e.g. different species of birds, deer, pets, other wildlife, and rodents. These sources have been shown over and over again to be major contributors to fecal loading in watersheds throughout Oklahoma and the United States. Additionally, the cattle manure composites were taken from 10 “patties” so it is impossible to know if one in ten or all ten in ten carried the biomarker. If only one in ten carried the biomarker, diluting it with 9 other patties may have reduced the concentration below the method detection limit. It is very probable that the bacterial communities of animals living in close proximity to one another will be similar. Therefore, in order to capture a more representative sample of the watershed, patties should have been taken from different farms, not all from the same farm (Hartel et. al, 2007, *Geographic sharing of ribotype*

patterns in enterococcus faecalis for bacteria source tracking). Furthermore, Dr. Harwood testified that the sample locations were not randomly selected and that the locations were chosen based on the Plaintiffs' belief that the results would be favorable to them. The Plaintiffs have not shown the 'biomarker' to be poultry specific because the sampling plan was biased and inadequate; not only were there an insufficient number of samples of the sources that were represented, many of the major sources were overlooked.

9.2.8. *Failure to demonstrate a relationship between the "biomarker" and pathogens:* The Plaintiffs claim that there is an imminent and substantial threat to public health from the land application of poultry litter. However, the Plaintiffs have failed to show that there is any relationship between the 'biomarker' and any pathogenic bacteria. There is no public health linkage between the biomarker and illness rates. No epidemiological studies have been conducted to determine if there is a correlation between these two parameters. The Plaintiffs did however, show that there are virtually no pathogens present (salmonella, campylobacter) in the waters of the IRW even though they claim that the "biomarker" is present.

9.2.9. *Failure to demonstrate a relationship between the "biomarker" concentrations and indicator bacteria concentrations in the IRW:* The Plaintiffs claim that the levels of biomarker can be used to quantify the indicator bacteria originating from poultry in the IRW. However, when developing the quantitative aspect of this assay, negative values were reported for the amount of total DNA present. It is impossible to have negative amounts of DNA. Therefore the standard curves used in quantification cannot be correct and the quantitative values reported by the Plaintiffs are also incorrect. In addition, the error rate of the qPCR was never considered. Finally, the Plaintiffs' proposed relationship between "biomarker" concentrations in the IRW versus the indicator bacteria

concentration is incorrect because the Plaintiffs made the incorrect assumption, as Plaintiffs' own scientists recognized, that all the indicator bacteria were derived from poultry, an assumption that is grossly inaccurate. Therefore, no risk assessments can be made nor is it possible to extrapolate the amount of indicator bacteria originating from poultry in the IRW.

9.2.10. Failure to follow all lab protocol: There were many procedural failures in the Plaintiffs' work. Samples were improperly labeled or misidentified e.g. was FAC01 or FAC1-6-20-06 litter or soil? Sample labels fell off during shipping and were reattached based on the best "guesses" by the receiving lab as to which sample coincided with which labels. Chain of Custody forms were missing, unsigned and potentially back dated. In a review of the emails provided by the Plaintiffs, it was obvious that the most basic record keeping, archiving, sample handling, chain of custody forms and standard laboratory procedures were not followed e.g., there were blanket sample receiving forms that indicated the samples were received in good conditions at the appropriate temperatures when in fact many were not. These parameters need to be checked each time samples are received, not listed by default as Ok on a blanket form. These and other errors cast doubt on the validity of the Plaintiffs' work.

9.2.11. Failure to utilize a standard method: The "poultry biomarker" MST method is not a standard method nor has it been peer reviewed or third party tested, making it at best a research method and not one that can or should be used for any regulatory action(s). Nor should it be used to draw conclusions about the sources of indicator bacteria in the IRW (or anywhere else). As discussed below, IEH conducted additional testing that that the Plaintiffs failed to do. This testing, which should have been undertaken, showed that the method was nonselective and nonspecific. This additional testing demonstrates the risk of

using an MST method before it has been properly validated. The field of MST has a history of methods being embraced, only to discover the methods' deficiencies following adequate validations. The Plaintiffs' consultants have recently submitted a draft regarding the development of this method to the American Society of Microbiology (ASM) journal *Applied and Environmental Microbiology*. This draft was fraught with data omissions and at best should conclude with "further study is needed". That further study was performed by IEH and unequivocally demonstrated that this method is not poultry specific.

9.3. IEH testing of the Plaintiffs' "biomarker":

9.3.1. IEH Laboratories followed the Plaintiffs' "biomarker" protocols (PCR and qPCR) and was able to successfully duplicate their methodology. In order to demonstrate that IEH had accurately reproduced the methodology, IEH tested pure cultures of *Brevibacterium casei* along with other *Brevibacterium sp.* The Plaintiffs reported that *Brevibacterium casei* produced positive signals both for the PCR and qPCR assays. IEH confirmed these results. As a secondary confirmation, IEH ran some litter, environmental, and plasmid DNA samples that the Plaintiffs provided. In addition, IEH evaluated the sensitivity of the assay by increasing the number of cycles.

9.3.2. IEH tested new samples:

9.3.2.1. Poultry litter and unused bedding material were supplied to IEH by Defendants. IEH extracted total DNA from both samples and ran the Plaintiffs' biomarker assays. The results were that both the PCR and qPCR assays were positive and generated the appropriate products from the poultry litter. IEH also enriched and incubated the unused bedding material and then extracted total DNA. The result was that that the LA35 PCR primer set generated the appropriate genetic "signature" from the bedding material. This means that the genetic material associated with the "biomarker",

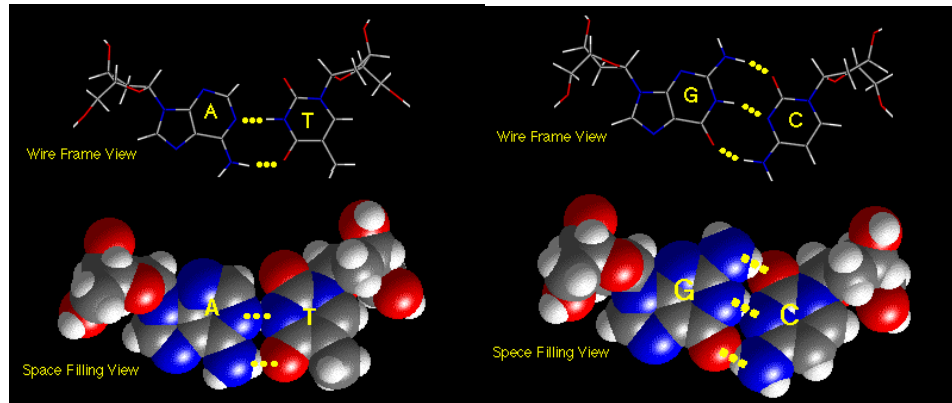
albeit in small quantities, was present in the bedding material prior to the introduction of the poultry.

9.3.2.2.IEH isolated over 375 cultures from litter and ran PCR on a subset of those cultures in an attempt to determine: 1) if the “biomarker” was derived from a viable organism, and 2) what that organism was. Isolates that gave positive PCR bands of the correct size were then run with qPCR and/or the PCR bands were sequenced. Isolates that generated bands that were not the correct size were set aside. Isolates that generated the correct size band included strains of: *Brevibacterium avium*, *Brevibacterium epidermidis*, *Corynebacterium ammoniagenes*, *Pantoea agglomerans*, *Exiguobacterium sp.*, and *Lysinibacillus sphaericus*. These organisms are not specific to poultry. It is important to note that when quantifying, qPCR does not distinguish band sequence or size, therefore the Plaintiffs’ qPCR assay will generate a positive result when any target is amplified, e.g., the two bands generated from *Brevibacterium casei* or primer dimer formation.

9.3.2.3.The Plaintiffs’ acknowledge that a positive qPCR may be generated by organisms with similar genetic sequences and/or samples with multiple sources of these organisms. In an attempt to address this issue the Plaintiffs’ claim that the qPCR melt curve will differentiate poultry specific signals from all others. This is simply not true. The melt curve does not differentiate poultry specific products. In the first place, the “biomarker” itself is not poultry specific. In the second, the melt curve is not sensitive enough to do so. DNA is double stranded; as a strand of DNA is heated the strands separate. The temperature at which 50% of the DNA becomes single stranded is defined as the melting temperature of that fragment of DNA. That temperature is a function of the length of the DNA strand and the composition of the

strand. The double strands are held together by bonds between the base pairs: adenine (A), cytosine (C), guanine (G) and thymine (T). These base pairs are complimentary; A will bind with T and C will bind with G. Two hydrogen bonds connect **FIGURE 5. Base Pair BONDING**

A



d

three connect G-C (**FIGURE 5**). Therefore, like sized strands with a higher G-C content will have a higher melting temperature than those with lower G-C contents

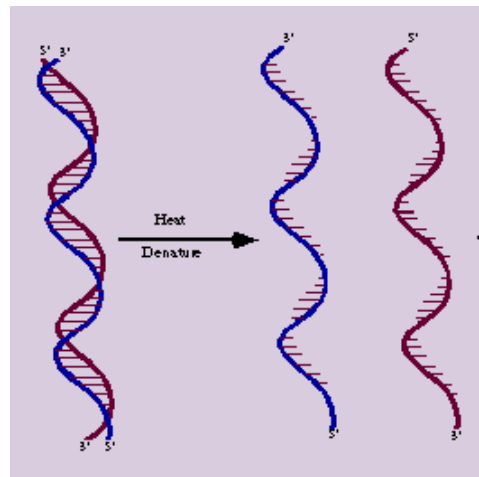


FIGURE 6. DNA, Double to Single Strands

because the energy (temperature) required to break three bonds is greater than that to break two. After qPCR, the products are heated so all the DNA is denatured

(**FIGURE 6**) – as this process is occurring the fluorescence is measured to determine

the melting temperature (**Figure 7**). Typically, this curve is not the one used to

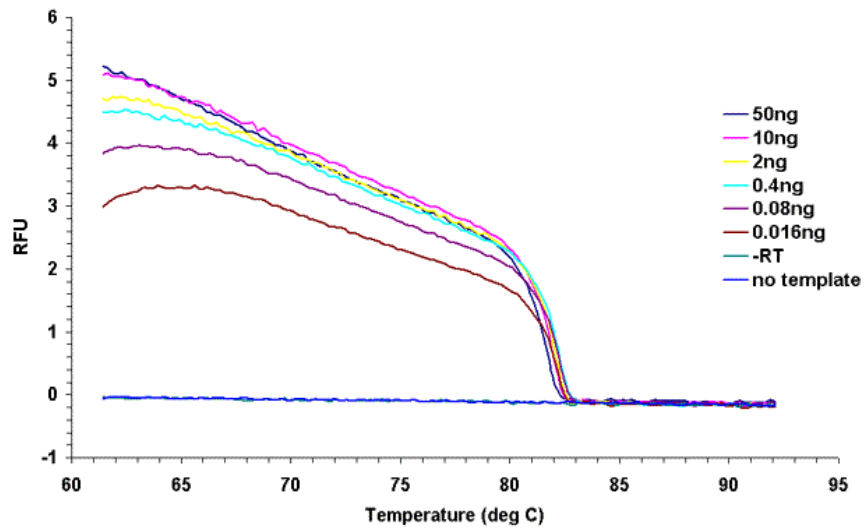


FIGURE 7. Melt Curve

represent the “melt curve”. Rather, the first derivative (dF/dT) of the initial curve is generated and the melting temperature is illustrated as the peak of this new curve as illustrated by **FIGURE 8**. This temperature and the shape of the melt curve are dependent on the conditions of the reaction and sample matrix and composition.

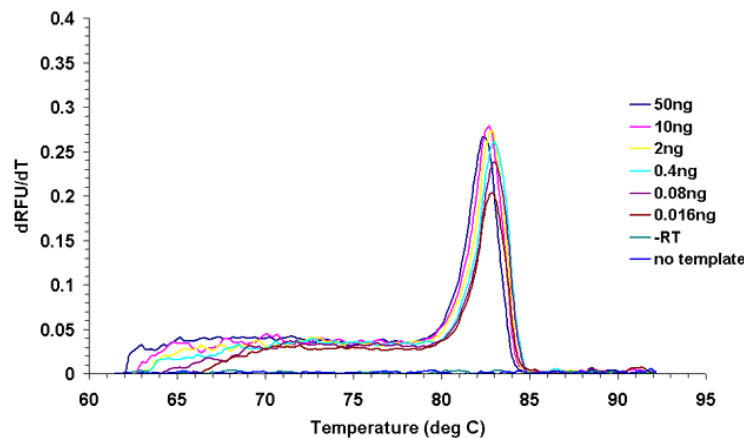


FIGURE 8. dF/dT ($dRFU/dT$) of “Melt Curve”

The solvent dimethyl sulfoxide (DMSO) is often added to PCR reactions to inhibit the formation of primer dimers or secondary structures although it increases the mutation rate within the PCR reaction (Chakrabarti R., Schutt C.E. (2001), "The enhancement of PCR amplification by low molecular-weight sulfones", *Gene* **274** (1-2):293-298)). The Plaintiffs' tried many different concentrations of DMSO to clean up their reaction and improve their melt curve. However, even under the same laboratory conditions, one technician generated primer dimers in his qPCR reactions while another technician did not. The shape of the melt curve graph is also a function of the software used to generate it. Depending on how many points are evaluated, the curve could be smoother or rougher or have one peak or many. Another factor affecting the shape of the curve is the concentration of the sample. Even with the same sample, different concentrations create slightly different melt curves (**FIGURE 7**). Three different isolates are represented in **FIGURE 9**; the melt curves all have approximately the same shape and the same melting temperatures.

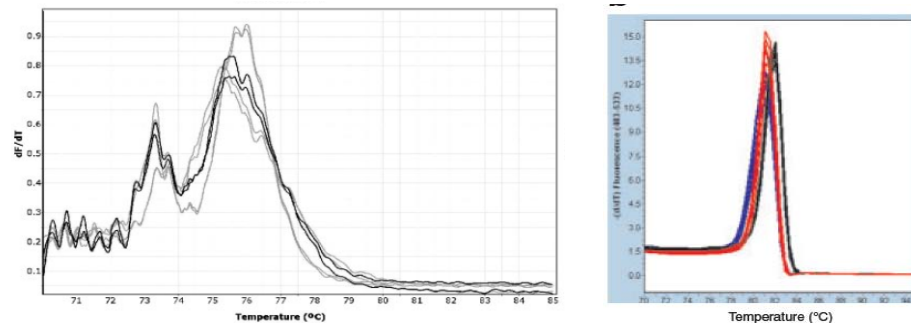


FIGURE 9. Two Sets of Melt Curves, Each Representing Three Unique Isolates

Using the melt curve analysis in the way the Plaintiffs' have (peak temperature), it would be impossible to determine that there were three separate isolates present. Although the melt curve is an excellent tool to determine if primer dimers or two

targets are present, it cannot determine the amount of each product that is present and is not sensitive enough to detect minor base pair differences in products that are of similar length. Therefore, the melt curve analysis does not differentiate poultry specific versus non poultry derived targets. It is important to note that there is a technique referred to as High-Resolution DNA Melt Curve Analysis. This technique is selective in applications such as single nucleotide polymorphism (SNPs) analysis. This technique is generally done with short fragment (~50 bp versus ~532 bp for the “biomarker”) where the position of the mutation is known so that a probe can be designed to target that portion of the DNA. In addition, the dF/dT curve is not used to differentiate the SNPs; the normalized difference curve is used. This is not the procedure the Plaintiffs used nor would it be feasible in the IRW because there are many potential sequences that produce similar sized products whose sequences are unknown.

9.3.2.4.IEH collected and tested 16 faecal samples from Canada Geese in the Seattle, WA area (Green Lake, etc.). The faecal samples were approximately 2 to 3 grams each and were collected with sterile cotton swabs, placed in sterile plastic bags and stored at 4°C. Subsequently, these samples were tested. The results were that all 16 of the Canada goose samples tested positive for the “biomarker”. (Plaintiffs’ own initial testing reflected a cow and two goose samples as “biomarker” positive. Subsequently produced documents show that a third goose sample was also positive.)

9.3.2.5.IEH collected sand samples from Juanita Beach, Kirkland, WA, a swimming beach on Lake Washington that was closed on 6/6/08 due to high levels of bacteria

(http://www.ci.kirkland.wa.us/News_Room/Juanita_Beach_Swimming_Area_Closed_Due_to_High_Bacteria_Levels.htm). There is no poultry production anywhere near Juanita beach however, there is a high population of waterfowl. The sand samples tested positive for the LA35 biomarker.

9.3.2.6.IEH routinely tests cow/cattle hide samples provided by various slaughter houses for pathogens (*E. coli* O157 and salmonella). Some of these samples were tested for the poultry “biomarker” and were positive. Unfortunately, because these samples tested negative for pathogens and were not collected specifically for this project, they were discarded. Recently, an additional cow hide sample was tested and it too was positive for the poultry “biomarker”. This sample has been appropriately archived.

9.3.2.7.IEH is involved in an MST Project (Blaine Harbor, WA) being conducted by the EPA.

As part of that project, Herrera Environmental Consultants collected known source faecal samples for library development on 9/10/2008. IEH received 4 samples: B7 - crow, C8 - dog, B9 and B10 - water fowl. The results were that both B9 and B10 were positive for the “biomarker”.

10. Conclusions

10.1.The current water quality standards for bacteria are insufficient to evaluate public health risks in mixed source watersheds. Indicator bacteria are ubiquitous and originate from multiple sources in the IRW. Major contributors include cattle, swine and wildlife.

10.2.There is no evidence of pathogen introduction or elevated levels of risk due to land application of poultry litter in the IRW. The plaintiffs' pathogen testing demonstrates the opposite – it shows that there are negligible if any levels of pathogens in the IRW that could potentially originate from poultry.

10.3.The fate and transport characteristics of the “biomarker” are unknown.

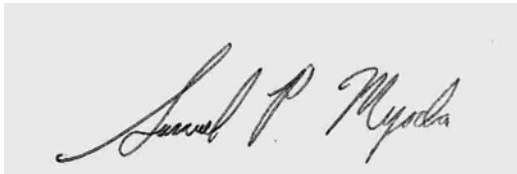
10.4.There is no statistically valid correlation between the “biomarker” concentrations v. indicator concentrations in the IRW.

10.5.The “biomarker” is neither selective nor specific and cannot be used as to qualify let alone quantify the source of the indicator bacteria in the IRW or if there is any effect on water quality due to the land application of poultry litter. The “biomarker” can be found in other sources such as ducks, geese, cattle, sand at beaches with no poultry influence and bedding material and no linkage to risk levels has been established nor has any valid correlation between indicators and “biomarker” concentration been developed. Using the Plaintiffs' methodology, IEH found the “biomarker” to be present in multiple sources AND also found that numerous organisms carry a genetic sequence that will yield positive results. Melt curve analysis is insufficient in distinguishing these other organisms because their sequences are too similar and/or the sample is a mixed culture.

10.6.There is no evidence that poultry is the major contributor of indicator bacteria into the waters of the IRW or that the land application of litter presents a public health threat in the IRW.

I declare under penalty of perjury that the foregoing is true and correct.

Executed on December 1, 2008

A handwritten signature in black ink on a light gray rectangular background. The signature is written in a cursive style and reads "Samuel P. Myoda".

Samuel P. Myoda

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FOUNDED 1866

February 23, 2009

David P. Page
Riggs, Abney, Neal, Turpen, Orbison & Lewis
502 West Sixth Street
Tulsa, OK 74119

Dear David:

I write with regard to Dr. Myoda's expert report. In reviewing the report we have identified a point requiring clarification. On page 33, in paragraph, 9.3.2.6, Dr. Myoda wrote: "Recently, an additional cow hide sample was tested and it too was positive for the 'biomarker.'" In fact, IEH tested four cow hide samples, of which two tested positive for the alleged biomarker and two tested negative. All four samples have been appropriately archived

Best regards,

A handwritten signature in black ink, appearing to read "J. Jorgensen".

Jay T. Jorgensen

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